

PCT

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁷ : G01N 33/50, A61K 49/00</p>	A3	<p>(11) International Publication Number: WO 00/31530</p> <p>(43) International Publication Date: 2 June 2000 (02.06.00)</p>																																																																							
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>(21) International Application Number: PCT/US99/27410</p> <p>(22) International Filing Date: 17 November 1999 (17.11.99)</p> <p>(30) Priority Data: 60/109,563 23 November 1998 (23.11.98) US</p> <p>(71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Bethesda, MD 20852 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): AMBS, Stefan [DE/US]; 455 Albermarle Road, Newtonville, MA 02460 (US). HARRIS, Curtis [US/US]; 4720 Waverly, Box 77, Garrett Park, MD 20896 (US).</p> <p>(74) Agents: PARENT, Annette, S. et al.; Townsend and Townsend and Crew LLP, 8th floor, Two Embarcadero Center, San Francisco, CA 94111-3834 (US).</p> </div> <div style="width: 48%;"> <p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p> <p>(88) Date of publication of the international search report: 21 September 2000 (21.09.00)</p> </div> </div>																																																																									
<p>(54) Title: SCREENING OF NOS2 MODULATORS USING P53 MUTANT CELLS</p> <table style="width: 100%; text-align: center; border-collapse: collapse;"> <thead> <tr> <th></th> <th colspan="2">RKO</th> <th colspan="2">HCT-116</th> <th colspan="2">HT-29</th> <th colspan="2">Calu-6</th> <th colspan="2">LoVo</th> </tr> <tr> <th></th> <th>BagLac Z</th> <th>NOS2 Cl. 5</th> <th>BagLac Z</th> <th>NOS2 Cl. 3</th> <th>BagLac Z</th> <th>NOS2 Cl. 1</th> <th>NOS2 Cl. 3</th> <th>BagLac Z</th> <th>NOS2 Cl. 5</th> <th>BagLac Z</th> <th>NOS2 Cl. 9</th> </tr> </thead> <tbody> <tr> <td style="text-align: left;">NOS2 —</td> <td colspan="11"></td> </tr> <tr> <td style="text-align: left;">Densitometry</td> <td></td> <td>14.6</td> <td></td> <td>13.3</td> <td></td> <td>9</td> <td>163</td> <td></td> <td>69</td> <td></td> <td>6.5</td> </tr> <tr> <td style="text-align: left;">VEGF —</td> <td colspan="11"></td> </tr> <tr> <td style="text-align: left;">Densitometry</td> <td>5.6</td> <td>6.2</td> <td>18</td> <td>33.6</td> <td>1.5</td> <td>1.2</td> <td>30.1</td> <td>1.5</td> <td>15</td> <td>4.8</td> <td>9.9</td> </tr> </tbody> </table>				RKO		HCT-116		HT-29		Calu-6		LoVo			BagLac Z	NOS2 Cl. 5	BagLac Z	NOS2 Cl. 3	BagLac Z	NOS2 Cl. 1	NOS2 Cl. 3	BagLac Z	NOS2 Cl. 5	BagLac Z	NOS2 Cl. 9	NOS2 —												Densitometry		14.6		13.3		9	163		69		6.5	VEGF —												Densitometry	5.6	6.2	18	33.6	1.5	1.2	30.1	1.5	15	4.8	9.9
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<p>(57) Abstract</p> <p>The invention provides, for example, <i>in vitro</i> and <i>in vivo</i> methods for screening modulators of NOS-2 activity using p53 mutant cells, methods of predicting the benefit of administering NOS-2 inhibitors to a cancer patient, and methods of treating cancer by administering NOS-2 inhibitors to patients with p53 mutant cancers.</p>																																																																									

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INTERNATIONAL COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 15280-358PC	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US99/27410	International filing date (day/month/year) 17/11/1999	Priority date (day/month/year) 23/11/1998
International Patent Classification (IPC) or national classification and IPC G01N33/48		
Applicant THE GOVERNMENT OF THE UNITED STATES OF AMERICA ...		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input checked="" type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 		
Date of submission of the demand 06/04/2000	Date of completion of this report 11.01.2001	
Name and mailing address of the international preliminary examining authority: <div style="display: flex; align-items: center;"> <div> European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 </div> </div>	Authorized officer Weaver, M Telephone No. +49 89 2399 8689	



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US99/27410

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).):*

Description, pages:

1-48 as originally filed

Claims, No.:

1-22 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US99/27410

- ☐ the drawings, sheets:
5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):
(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:
- ☐ the entire international application.
- ☒ claims Nos. 19, 20 (part), 21, 22.

because:

- ☒ the said international application, or the said claims Nos. 19, 20 (part), 21, 22 with respect to industrial applicability relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☐ no international search report has been established for the said claims Nos. .
2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US99/27410

Novelty (N)	Yes: Claims 1-22
	No: Claims
Inventive step (IS)	Yes: Claims
	No: Claims 1-22
Industrial applicability (IA)	Yes: Claims 1-18; 19, 20 (part)
	No: Claims

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Section III

Claims 19 and 20, insofar as they encompass an in vivo diagnostic method, and claims 21 and 22 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Section V

1. D1 (Oncogene 17 (1998) 2323; abstract; page 2323, column 1, lines 27 - 40; page 2327, column 1, lines 16 - 46; page 2329, column 2, lines 9 - 17) discloses that inducible nitric oxide synthase (iNOS) is under negative regulatory control of p53 and that enhanced expression of iNOS occurs in cells expressing a mutant p53 protein. Various modulators of iNOS are also disclosed.
2. D2 (Proc Natl Acad Sci USA 95 (1998) 8823; abstract; page 8823, column 1, line 1 - column 2, line 33) also discloses that p53 protein accumulation down-regulates iNOS (referred to here as NOS2) and that NOS2 expression is up-regulated in p53 knockout mice. Stimulators and inhibitors of NOS2 are also identified.
3. Thus, although there is no explicit disclosure of the method of claim 1 of the present application in either of D1 or D2, it would be immediately apparent to the skilled person reading either of these documents that, in order to screen for modulators of NOS2 other than those described in D1 or D2, a cell system that expresses NOS2, such as the p53 mutant-expressing cells described in D1 or D2, whether in vitro (e.g. glioma cells as in D1) or in vivo (e.g. p53 knockout mice as in D2) would be the only and obvious choice when seeking to solve the problem of providing a means of identifying new NOS2 modulators.

Claim 1 thus lacks an inventive step and does not meet the requirements of Article 33(3) PCT.

4. Dependent claims 2 - 10 do not appear to contain any features which, in combination with the features of any claim to which they refer, meet the

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US99/27410

requirements of the PCT in respect of inventive step, given the disclosures of D1 and D2.

5. As the assays described in D2 are also performed in vivo in mice (see point 3 above), claims 11 - 18 also lack an inventive step (Article 33(3) PCT) for the reasons set out in point 3.
6. The relationship between NO, NOS2 and p53 expression is described in D1 and D2. Moreover, D2 (in particular page 8823, column 2, lines 22 - 27; page 8827, column 1, lines 3 - 32) suggests in very clear terms that increased NO levels produced by NOS2 in p53 knockout mice may cause cancer progression in some cell types, such as lymphocytes, which have a loss of p53 function.

In the light of this disclosure in D2, the objective problem to be solved by the subject-matter of claims 19 and 21 is considered to be the provision or identification of means for reducing NO levels in order to treat cancer progression in p53 mutant cancer cells. This problem is solved by identifying and/or administering a NOS2 inhibitor. However, NOS inhibition and inhibitors are known in the art (see e.g. D2, page 8823, column 2, lines 4 - 8). This solution is therefore merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed.

Claims 19 and 21 and their dependent claims 20 and 22 therefore do not meet the requirements of Article 33(3) PCT.

7. For the assessment of the present claims 19 and 20, insofar as they encompass an in vivo diagnostic method, and claims 21 and 22 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US99/27410

8. It should be noted that as a result of the language "immune deficient animal" used in claim 11, claims 11 - 17 encompass a screening method performed on living human subjects. Such methods are unacceptable in most jurisdictions (e.g. that of the EPO) as being contrary to morality.

Section VII

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 is not mentioned in the description, nor is this document identified therein.

Section VIII

1. The meaning of the abbreviation "NOS2" (page 7, line 33 of the description) would have to be given at least once in the claims (Article 6 PCT).
2. The term "p53 mutant cell" is vague and unclear (Article 6 PCT) and would have to be replaced by the definition given on page 7, lines 24/25 of the description.

PATENT COOPERATION TREATY

ASP

From the

INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

TOWNSEND & TOWNSEND
& CREW

To:

WEBER, Kenneth A.
TOWNSEND AND TOWNSEND AND CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, CA 94111-3834
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01 JAN 23 AM 10:44

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NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
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(PCT Rule 71.1)

Date of mailing
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11.01.2001

Applicant's or agent's file reference
15280-358PC

IMPORTANT NOTIFICATION

International application No.
PCT/US99/27410 ✓

International filing date (day/month/year)
17/11/1999 ✓

Priority date (day/month/year)
23/11/1998 ✓

Applicant

THE GOVERNMENT OF THE UNITED STATES OF AMERICA ... ✓

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

5/23/01

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

Pedersen, C

Tel. +49 89 2399-8063



Km

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

To:
TOWNSEND AND TOWNSEND AND CREW LLP
Attn. WEBER, K.A.
Two Embarcadero Center, 8th Floor
San Francisco, CA 94111-3834
UNITED STATES OF AMERICA

Date of mailing
(day/month/year) 04/07/2000

Applicant's or agent's file reference
15280-358PC

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/US 99/ 27410 ✓

International filing date
(day/month/year) 17/11/1999 ✓

Applicant
THE GOVERNMENT OF THE UNITED STATES OF AMERICA ...

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.
Filing of amendments and statement under Article 19: 9-4-00
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.


2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.
☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later). 5-23-01
7-23-00 (undocketed)
Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II. ch. 2 6-23-00 (undocketed)

<p>Name and mailing address of the International Searching Authority</p> <p> European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016</p>	<p>Authorized officer</p> <p>Jaap Hurenkamp</p>
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NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 15280-358PC	FOR FURTHER ACTION		see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US 99/ 27410	International filing date (day/month/year) 17/11/1999	(Earliest) Priority Date (day/month/year) 23/11/1998	
Applicant THE GOVERNMENT OF THE UNITED STATES OF AMERICA ...			

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

SCREENING OF NOS2 MODULATORS USING P53 MUTANT CELLS

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

6

☒ as suggested by the applicant.

☐ None of the figures.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International Application No

PO 99/27410

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 G01N33/50 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>RIGER JOHANNES; STAENDER MARKO; LOESCHMANN PETER A; HENKA MICHAEL; DICHGANS JOHANNES; KLOCKGETHER THOMAS; WELLER MICHAEL: "Synthesis and biological effects of NO in malignant glioma cells: Modulation by cytokines including CD95L and TGF-beta, dexamethasone, and p53 gene transfer" ONCOGENE, vol. 17, 5 November 1998 (1998-11-05), pages 2323-2332, XP000920683 page 2327</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-22



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

20 June 2000

Date of mailing of the international search report

04/07/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
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 Fax: (+31-70) 340-3016

Authorized officer

Hart-Davis, J

INTERNATIONAL SEARCH REPORT

International Application No

PC 99/27410

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AMBS STEFAN; OGUNFUSIKA MOFULUSARA O; MERRIAM WILLIAM G; BENNETT WILLIAM P; BILLIAR TIMOTHY R; HARRIS CURTIS C: "Up-regulation of inducible nitric oxide synthase expression in cancer-prone p53 knockout mice" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 95, July 1998 (1998-07), pages 8823-8828, XP002140548 cited in the application the whole document	1-22
A	AMBS STEFAN; HUSSAIN S PERWEZ; HARRIS CURTIS C: "Interactive effects of nitric oxide and the p53 tumor suppressor gene in carcinogenesis and tumor progression" FASEB JOURNAL, vol. 11, May 1997 (1997-05), pages 443-448, XP002140549 cited in the application the whole document	1-22
P,X	AMBS STEFAN; MERRIAM WILLIAM G; OGUNFUSIKA MOFULUSARA O; BENNETT WILLIAM P; ISHIBE NAOKO; HUSSAIN P PERWEZ ET AL.: "p53 and vascular endothelial growth factor regulate tumor growth of NOS2-expressing human carcinoma cells" NATURE MEDICINE, vol. 4, December 1998 (1998-12), pages 1371-1376, XP002140550 page 1373	1-22
P,X	SHI QIAN; HUANG SUYUN; JIANG WEIDONG; KUTACH LAURA S; ANANTHASWAMY HONNAVARA N; XIE KEPING: "Direct correlation between nitric oxide synthase II inducibility and metastatic ability of UV-2237 murine fibrosarcoma cells carrying mutant p53" CANCER RESEARCH, vol. 59, 1 May 1999 (1999-05-01), pages 2072-2075, XP002140551 abstract page 2073; table 1	1-22

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INTERNATIONAL SEARCH REPORT

International Application No

PC 99/27410

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>MATSUMOTO HIDEKI; HAYASHI SACHIKO; HATASHITA MASANORI; OHNISHI KEN; OHTSUBO TOSHIO; KITAI RYUHEI; SHIOURA HIROKI ET AL.: "Nitric oxide is an initiator of intercellular signal transduction for stress response after hyperthermia in mutant p53 cells of human glioblastoma" CANCER RESEARCH, vol. 59, 1 July 1999 (1999-07-01), pages 3239-3244, XP002140552 abstract</p> <p>---</p>	1-22
P,A	<p>AMBS STEFAN; BENNETT WILLIAM P; MERRIAM WILLIAM G; OGUNFUSIKA MOFOLUSARA O; OSER SEAN M; HARRINGTON ANITA M ET AL.: "Relationship between p53 mutations and inducible nitric oxide synthase expression in human colorectal cancer" JOURNAL OF THE NATIONAL CANCER INSTITUTE (BETHESDA), vol. 91, 6 January 1999 (1999-01-06), pages 86-88, XP000920692 the whole document</p> <p>---</p>	1-22
A	<p>MESSMER UDO K; BRUENE BERNHARD: "Nitric oxide-induced apoptosis: p53-dependent and p53-independent signalling pathways" BIOCHEMICAL JOURNAL, vol. 319, 1996, pages 299-305, XP000920872 cited in the application the whole document</p> <p>---</p>	1-22
A	<p>AMBS STEFAN; MERRIAM WILLIAM G; BENNETT WILLIAM P; FELLE-BOSCO EMANUELA; OGUNFUSIKA MOFOLUSARA O; OSER SEAN M ET AL.: "Frequent nitric oxide synthase-2 expression in human colon adenomas: Implication for tumor angiogenesis and colon cancer progression" CANCER RESEARCH, vol. 58, 15 January 1998 (1998-01-15), pages 334-341, XP002140553 cited in the application the whole document</p> <p>---</p>	1-22
A	<p>AMBS S; BENNETT W P; MERRIAM W G; OGUNFUSIKA M O; OSER S M; KHAN M A; JONES R T; HARRIS C C: "Vascular endothelial growth factor and nitric oxide synthase expression in human lung cancer and the relation to p53" BRITISH JOURNAL OF CANCER, vol. 78, July 1998 (1998-07), pages 233-239, XP000920696 the whole document</p> <p>---</p>	1-22

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INTERNATIONAL SEARCH REPORT

International Application No

PC 99/27410

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>FORRESTER KATHLEEN; AMBS STEFAN; LUPOLD SHAWN E; KAPUST RACHEL B; SPILLARE ELISA A; WEINBERG WENDY C ET AL.: "Nitric oxide-induced p53 accumulation and regulation of inducible nitric oxide synthase expression by wild-type p53" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 93, March 1996 (1996-03), pages 2442-2447, XP002140554 cited in the application the whole document</p> <p>-----</p>	1-22

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 11-18 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. Although claims 21-22 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/27410

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.